

**STUDIES ON EXO- AND ENDO-ENZYMES OF SELECTED
STRAINS OF *Bacillus thuringiensis* WITH RESPECT TO
INSECTICIDAL ACTIVITY**

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ABSTRACT

Bacillus thuringiensis is being used widely as a biological control agent against insects. This bacterium produces a parasporal crystal consisting of a delta endotoxin which is toxic to larvae of Lepidoptera, Diptera, and Coleoptera. An attempt was made to study the relationship between selected enzymes produced by local isolates to spore and crystal formation in the bacteria. Protease, alkaline phosphatase, pyruvate carboxylase, isocitrate dehydrogenase and citrate synthase were monitored at various stages of growth in fermentation media. The high activity of isocitrate dehydrogenase for IPT BT 6 could be used as an useful tool to identify IPT BT 6 and also proves that it is a different strain from Florbac.

Crystals were purified from spore-crystal complex and molecular weights were determined by SDS-PAGE. IPT Bt 6, IPT Bt 15 and Florbac exhibited polypeptides with molecular weights of 56 000 and 120 000 Dalton; Florbac at 92 000, 73 000 and 39 000; IPT Bt 15 at 120 000 and 56 000; IPT Bt 16 at 56 000, 39 000 and 19 000 while IMR Bt 8 and IMR Bt 16 at 80 000 and also at 45 000 Dalton.

Florbac and IPT Bt 6 were selected for scale-up studies in B Braun fermentors. 1.5 litres was scaled up to 10 litres. Process optimization was carried out to achieve maximum product yield. The efficacy of the two isolates was assessed against *Plutella xylostella* known as the Diamond back moth which is a pest of cabbage. The activity was compared in various combination against a commercial product (Agrimec). IPT Bt 6 and Agrimec were active against *P. xylostella* while the local population had built up resistance to Florbac.

LIST OF ABBREVIATIONS

°C	Degree celsius
µg/ml	Microgram/millilitre
BSA	Bovine serum albumin
cm	centimeter
CsCl	Caesium chloride
DNS	Dinitrosalicylic acid
DPA	Dipicolinic acid
DTNB	Dithiobis-(2-Nitrobenzoic acid)
DTT	Dithiothreitol
EDTA	Ethylenediamine tetra-acetate
FeSO ₄	Ferrous sulfate
g	Gram
g/L	Grams/Litre
HCl	Hydrochloric acid
IMR	Institute for Medical Research
K ₂ HPO ₄	Dipotassium hydrogen phosphate
KH ₂ PO ₄	Monopotassium dihydrogen phosphate
L	Litre
LC ₅₀	Concentration of toxin which kills 50% of a given insect larvae population at a particular time
MARDI	Malaysian Agriculture Research and Development Institute

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MgSO ₄	Magnesium sulfate
mg/L	Milligram/Litre
mg/ml	Milligram/millilitre
min	Minute
ml/L	Millilitre/Litre
MnSO ₄	Manganese sulfate
NaCl	Sodium chloride
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium hydroxide
nm	Nanometer
nmol	Nanomol
OD	Optical density
<i>orf</i>	Open reading frame
PHB	Poly-β-hydroxybutyrate
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate Polyacrylamide gel electrophoresis
TEMED	Tetra methyl-ethylenediamine
w/v	Weight/volume

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